CHAPTER 2

Literature Review

2.1 Introduction

Pineapple (*Ananas comosus* (L) Merr.) is a terrestrial perennial monocotyledonous herb belonging to the family *Bromeliaceae* which embraces about 2,000 species (Coppens d'Eeckenbrugge and Leal, 2003). The mature pineapple plant is 1–2 m high and wide, and it is inscribed in the general shape of a spinning top. Pineapple fruit develops from multiple flowers fused together on the same inflorescence (peduncle), so it is identified in a multiple fruit group. Each ovary of flower develops on the peduncle. Moreover there are a cluster of leaves the top of the fruit the called crown, the morphological structures of pineapple fruit are shown in Figure 2.1.

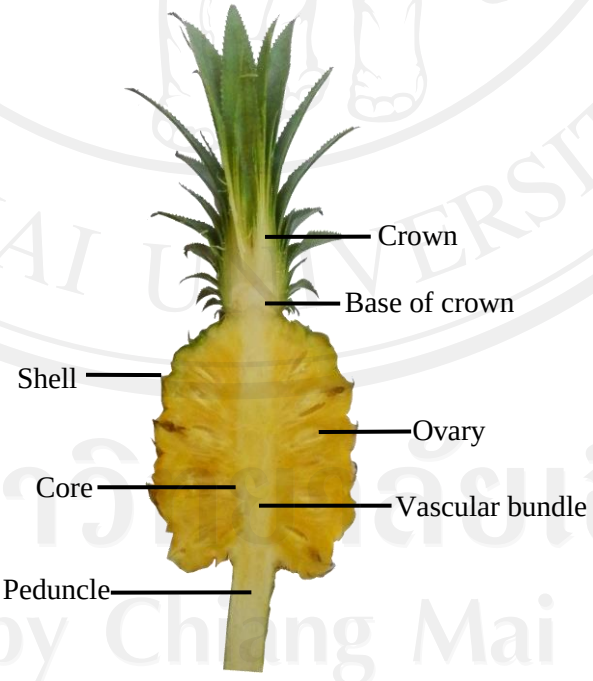

Figure 2.1 Morphological structures of pineapple fruit

Pineapple composition has been investigated mainly in the edible portion. Reported ranges of the main components from data collected from several commercial operations and include additional variables as environmental factors and degree of maturity of the fruit. Pineapples contain 81.2 to 86.2% moisture, and 13-19% total solids, of which sucrose, glucose and fructose are the main components. Carbohydrates represent up to 85% of total solids whereas fiber makes up for 2-3%. Of the organic acids, citric acid is the most abundant. The pulp has very low ash content, nitrogenous compounds and lipids (0.1%). From 25- 30% of nitrogenous compounds are true protein. Out of this proportion, ca. 80% has proteolytic activity due to a protease known as Bromelin (Dull, 1971). Fresh pineapple contains minerals as calcium, chlorine, potassium, phosphorus and sodium. Table 2.1 describes the overall composition of fresh pineapple.

Table 2.1 Chemical composition of pineapple fruit (Dull, 1971)

Component	%, wet basis of edible portion
Total soluble solids (TSS)	10.8-17.5
Titratable acidity (as citric acid)	0.6-1.62
Ash	0.3-0.42
Moisture	81.2-86.2
Fiber	0.3-0.61
Lipids	0.2
Esters (ppm)	1-250
Pigments (ppm carotenes)	0.2-2.5
Total nitrogen	0.045-0.115
Protein	0.181
Soluble nitrogen	0.079
Ammonia	0.010
Total amino acids	0.331

2.2 Pineapple production in Thailand

Based on the plant habit, especially the shape of leaf and fruit, pineapple crop is grouped into five groups (Collins 1968). These 5 groups are Cayenne, Spanish, Queen, Pernambuco and Mordilona. Commercial production is based mainly on clones in the 'Cayenne' group, also known as 'Smooth Cayenne'. The popular cultivars of pineapple in Thailand are the following (Youryon, 2011):

Cayenne Group is mostly used for canning and fresh fruit. The leaf edges are looking smooth. The fruit are ovoid medium-size fruit (1.5 - 2.5 Kg) on the strong peduncle. When the fruit ripens, the shell colorations turns yellow from the base to the top. The fresh is yellow, soft and juicy. The production cycle of Smooth Cayenne is longer than that of most other cultivars. Local cultivars are such as 'Pattavia' and 'Nang Lae'.

Spanish Group is medium-size (1.0 – 1.5 Kg) of fruit and orange with a round shape 'Intrachit kow' and 'Intrachit dang' are cultivated pineapple in this grown which were popular in the past. It has small, oval to cylindrical-shaped, and dark purple fruits that will turn copper-orange when ripening. The flesh is golden- yellow, low sugar and acidity, and poor in taste. The leaf spines are varied from clone to clone (Popluechai *et al.*, 2007).

Queen Group is suitable for fresh consumer. The plant is small, comprising short spiny leaves and a small fruit (0.5 – 1 Kg), while the shell turns full yellow with small prominent eyes. The pulp is golden-yellow, crispy, and sweet with an excellent flavor and long shelf life. Queen is tolerant to stress, pests and diseases but susceptible to chilling and internal browning. Some Thai cultivars include 'Phuket' 'Trad Srithong' 'Sawi' and 'Phu Lae'.

Pernambuco Group has an erect growth habit, semi-vigorous with long spiny leaves. The fruits are small, slender and carried on long peduncles. The flesh is white, tender and juicy with low acid and mild flavour. Slips are prominent and numerous, occasionally to the extent of hiding the fruit.

Mordilona Group is typified by the 'piping' leaf margin where a part of the lower epidermis folds over the edge of the leaf to give a completely spineless leaf form. The irregular, cylindrical fruit is large (1.5 - 3 kg) with attractive yellow to orange peel borne on a long peduncle. The flesh is cream to yellow, firm and sweet. Numerous crownlets protrude from the base of the crown and the upper eyes and slips are numerous.

Phulae pineapple refers to the Queen group. The fruit has cylindrical shape and small, weighing 0.15 - 1 Kg (the size of the fruit is approximately 7 cm in diameter and 9 cm in length). It is smaller than other varieties of pineapple (Figure 2.2). The young fruit has a green skin; the skin is rather thick and suitable for long distance transport. When it is ripe, the skin becomes orange-yellow. The pale yellow flesh is crispy, fragrant, and sweet. The core is crispy and edible. It is popularly eaten fresh and grown in the northern of Thailand, especially in Chiang Rai province. It propagates by separation of young shoots or growth buds.

Figure 2.2 Phu Lae pineapples

2.3 Harvesting, postharvest handling and postharvest diseases

2.3.1 Harvesting and handling

Pineapple fruit maturity is evaluated on the extent of fruit "eye" flatness and skin yellowing. Consumers similarly judge fruit quality by skin color and aroma. A fruit do not continue to ripen or sweeten after harvest. Immature fruit should not be shipped, since they do not develop good flavor, have low brix, and are more prone to chilling injury (Rohrbach and Paull, 1982). Pineapple fruit should be broken off the stalk with a downward motion, or cut with a knife slightly below the base of the fruit. The crown should be left intact and protected from damage. Removal of the crown increases the risk of disease and decay and is not recommended. The fruit should be moved as soon as possible to a shaded collection area or packing facility. Fruit left in the direct sun will sunburn and turn soft. The damaged tissue is more susceptible to postharvest decay.

Australia permits the importation of fresh pineapple fruit from the Philippines, Thailand, Sri Lanka and Solomon Islands, subject to a range of phytosanitary measures. Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia. Thus, they are recommended a risk management measures and operational systems that will reduce the risk associated with the importation of fresh pineapple fruit from another country into Australia. The postharvest handling for fresh pineapple is recommended following;

1) Sorting and de-crowning: fruit is sorted manually into export quality. Fruit that is rotten or heavily infested is discarded. The pineapple crown is removed and the stalk is trimmed to meet the importing country’s conditions.

2) Pest inspection: fruit is inverted over a container of pesticide Decis 205 (active ingredient deltamethrin 2.8%) and tapped firmly to remove any pest contaminant.

3) Fungicide treatment: fruit stems are treated immediately after sorting and pest inspection with an anti-fungal treatment.

4) Cleaning: fruit is cleaned using an air blower to remove any remaining pest or soil debris.

5) Grading and packing: suitable fruit are selected for export, weighed and packed into corrugated fiber board cartons. Cartons are placed on pallets inside containers.

6) Fumigation/post-harvest treatment: fruit would be treated using an approved product/methodology. Fumigation treatments can only be conducted by providers registered under the Australian Fumigation Accreditation Scheme (AFAS).

The process of cleaning, sorting, fungicide treatment, weighing, grading and packaging is carried out manually (Figure 2.3).

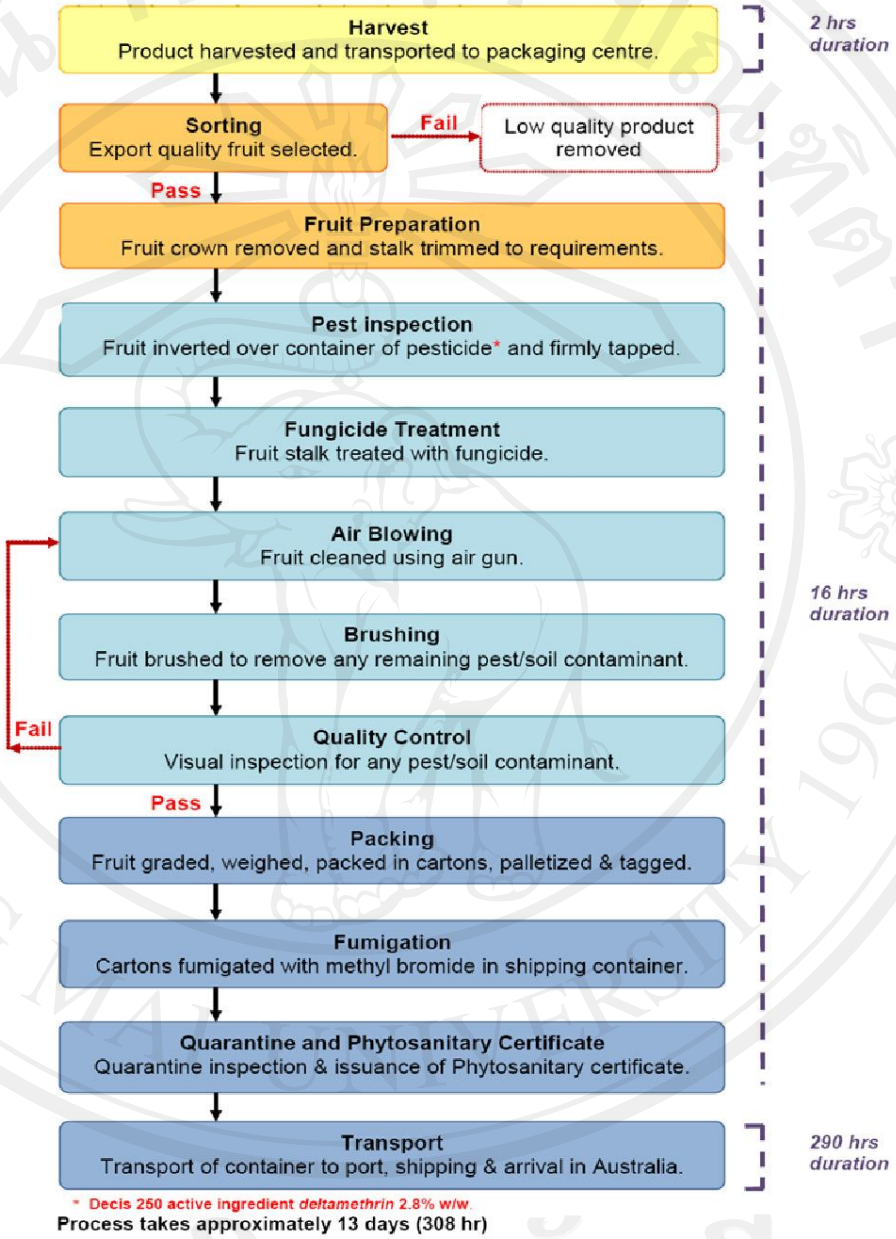

Figure 2.3 Exportation activities of fresh pineapple to Australia(based on a consignment with a 30 tonne capacity) (Department of Agriculture, Fisheries and Forestry, Australia, 2012).

2.3.2 Storage

Temperatures of 7 - 12 °C are recommended for storage of pineapples for 14 - 20 days, provided fruit are at the color break stage. A relative humidity (RH) of 85 to 95% is recommended; a high RH significantly reduces water loss. Ripe fruit can be held at 7.2 °C for about 7 to 10 days. Pineapples may be stored at 0 to 4 °C (32 to 39 °F) for weeks, but upon removal, fruit fail to continue ripening and show severe chilling injury. Quarter-yellow fruit at harvest gain about one additional week of storage for every 6 °C decrease in storage temperature (Dull, 1971). The maximum storage-life at 7 °C is about 4 weeks (Paull and Rohrbach, 1985). However, when removed, chilling injury-induced internal browning develops within 2 to 3 days.

2.3.3 Quality

Pineapple is a non-climacteric fruit. As a non-climacteric fruit, obvious compositional changes after harvest are mostly limited to degreening and decrease in acidity. No quality improvement can be expected after harvesting. Therefore, harvesting at the correct stage of maturity is essential. Because of the inability to develop quality after harvesting, fruit must be allowed to remain in the plant until they have attained satisfactory eating quality. A non-climacteric fruit harvested when fully mature is immediately edible, and does not undergo comparable ripening process, either on or off the plant (Kader, 1992).

Texture, color and nutrients constitute the critical quality attributes of fruits and vegetables. Since fruits picked from the whole plants, they went to ripening, senescence and death gradually, followed by the deterioration of texture and color, and the decrease of nutrients. Texture not only connects with the edible quality of products, but also is an indicator of storage property and effect. Firmness is a visual trait that directly represents the texture of fruits and vegetables. Due to the physiological activities such as respiration and transpiration, firmness of fresh products decreases gradually during storage, largely influencing quality and facilitating pathogen infection. Color is an important sensory quality of fresh fruits and vegetables and depends on the pigments they contain (Chen and Zhu, 2011).

Nutritional components, it is well known that fruits and vegetables are the main dietary source of vitamin C, and also are rich in many other nutritional components such as polyphenol, flavonoid, saccharides and organic acids. Thus they are functional in keeping people healthy and have specific flavors. In recent years, many researchers explored the maintenance of these nutritional components through ultrasonic pretreatment before storage (Chen and Zhu, 2011; Cao *et al*., 2010). In these researches, contents of vitamin C, total flavonoids, total phenols, total soluble solids (TSS), reducing sugars and titratable acidity (TA) were compared between several types of fruits/vegetables with and without ultrasonic treatment. Results showed that these components were maintained at higher levels in ultrasound treated fresh products.

2.3.4 Postharvest disease

Many fruit disease symptoms have been described on pineapple. Decay is significantly higher in mechanically injured fruit, when poor sanitation practices are followed, or if postharvest cooling is not provided. The fused nature of the fruitlets means that the flesh of the fruit is not sterile and contains yeasts and bacteria. Specific pathogens followed by massive infection by broader spectrum secondary invader are also reported (Collins, 1968). The most common ones are black rot, fruitlet core rot, and yeast fermentation.

Black rot, also called *Thielaviopsis* fruit rot, water blister, soft rot, or water rot, is a universal fresh pineapple problem characterized by a soft watery rot. Diseased tissue turns dark in the later stages of the disease because of the dark mycelium and spores(Figure 2.4). Black rot is caused by the fungus *Chalara paradoxa* (De Seynes) Sacc. Red Spanish types are more resistant than 'Smooth Cayenne.' Infection occurs within 8 to 12 h following harvest and enters through the point of detachment or wounds. The severity of the problem is dependent on the degree of bruising or wounding during harvesting and packing, the level of inoculum on the fruit, and storage temperature during transportation and marketing. The rot is commercially controlled by minimizing bruising of fruit during harvest and handling, refrigeration, and postharvest fungicides (Rohrbach and Phillips, 1990).

Fruitlet core rot, black spot, fruitlet brown rot, and eye rot describe the brown to black color of the central part of an individual fruitlet (Figure 2.5). Epidemic levels are rare in the major commercial pineapple-producing areas of the world. Low-acid cultivars being grown commercially are most susceptible. This disease is caused by a complex of fungi. Infection frequently can lead to misshapen fruit that are culled before packing and shipping (Rohrbach and Schmitt, 2003).

Yeasty fermentation arises due to the fact that fruit are not sterile inside, containing many non-growing, but viable yeasts and bacteria. In damaged, overripe fruit and fruit with inter-fruitlet cracking, resident yeasts begin to grow, or new yeasts invade. This growth leads to fermentation and bubbles of gas and juice through cracks in the skin. The skin turns brown and leathery and fruit become spongy with bright yellow flesh (Figure 2.6).

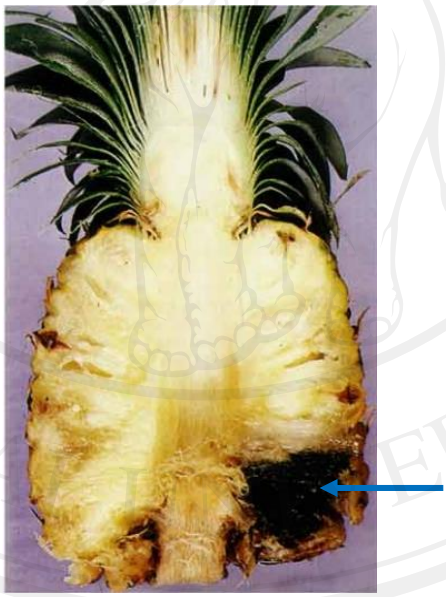

Figure 2.4 Black-rot or soft-rot symptoms on pineapple fruit (http://nhb.gov.in/fruits/pineapple/pin002.pdf)

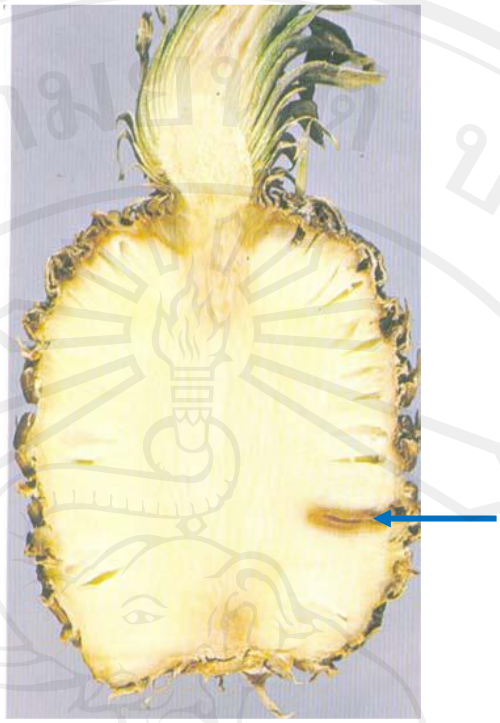

Figure 2.5 Symptoms of fruitlet core rot on pineapple (Ministry of Fisheries, Crops and Livestock, 2002)

Figure 2.6 Pineapple fruit affected by yeasty fermentation (Ministry of Fisheries, Crops and Livestock, 2002)

2.4 Strategies for postharvest disease control

Postharvest diseases cause considerable losses to harvested fruits. Control of postharvest diseases of fruit is mostly dependent on control of storage atmosphere, refrigeration and fungicides (Kader, 1992).

Fungicides: Postharvest fungicides can be applied as dips, sprays, fumigants, treated wraps and box liners or in waxes and coatings. Dips and sprays are very commonly used and depending on the compound, can take the form of aqueous solutions, suspensions or emulsions. Fungicides commonly applied as dips or sprays include the benzimidazoles (e.g. benomyl and thiabendazole) and the triazoles (e.g. prochloraz and imazalil). The benzimidazole group of fungicides are very useful for the control of many important postharvest pathogens such as *Pennicillium* and *Collectrotichum* Fumigants, such as sulphur dioxide for the control of grey mold (caused by *Botrgtis cinerea*) of grape and various postharvest diseases of lychee, are sometimes used for disease control (Coates and Johnson, 2013).

Low temperature storage: Temperature is so critical to postharvest disease control that all other treatments can be considered as supplements to refrigeration. Fruit rot fungi generally grow optimally at 20 - 25 ºC (68 - 77 °F) and can be conveniently divided into those with a growth minimum of 5 - 10 ºC (41 - 50 °F), or -6 - 0 ºC (21.2 - 32 °F). Fungi with a minimum growth temperature below -2 ºC (28.4 °F) cannot be completely stopped by refrigeration without freezing fruit. However, temperatures as low as possible are desirable because they significantly slow growth and thus reduce decay (Peter *et al.*, 2013).

Heat treatment: Hot water treatment of fruit following harvest has been demonstrated to protect horticulture produces against postharvest decay (Ferguson *et al.*, 2000; Lana *et al*., 2005). The mode of action of the hot water treatments may be through the impact of the heat to kill the pathogens directly or indirectly on horticulture produces (Lurie *et al.*, 1996). Hot air treatment has been used to control decay in crops that are injured by hot water. Heating of pears at temperatures from 21 - 38 ºC (69.8 - 100.4 °F) for 1 - 7 days reduced postharvest decay (Spotts and Chen, 1987). Recently, Wijeratnam *et al*. (2005)

reported that pineapples inoculated with 10^4 spores/ml, *C. paradoxa,* followed by a hot water dip treatment at 54 ◦C for 3 min were free of disease when stored at 10 ◦C for 21 days followed by 48 h at an ambient temperature (28±2 ◦C).

Controlled atmosphere storage: Alterations in O_2 and CO_2 concentrations are sometimes provided around fruit and vegetables. With closed control of these gases, the synthetic atmosphere is commonly called a controlled atmosphere; the term modified atmosphere is used when there is little possibility of adjusting gas composition during storage or transportation. Because the pathogen respires as does produce, lowering the O_2 or raising the CO_2 above 5% can suppress pathogenic growth in the host. Low O_2 does not appreciably suppress fungal growth until the concentration is below 2%. Important growth reductions result if the $_{O2}$ is lowered to 1% or lower although there is a danger that the crop will start respiring anaerobically and develop off-flavor (Peter *et al.,* 2013).

Biological control: Postharvest biological control is a relatively new approach and offers several advantages over conventional biological control (Alvindia and Natsuaki, 2008). The main modes of action of the biocontrol agent include competition for nutrients (Altintas and Ugur, 2008) and space (Djonovic *et al.*, 2007), production of cell wall degrading enzymes and production of antifungal diffusible and volatile metabolites (Tahia *et al.,* 2004). Pineapple biological control treatment have been reported by Wijesinghe *et al.,* 2010 who found that pineapple fruits inoculated with 10^5 conidia/mL of *Thielaviopsis paradoxa*, followed by an application of a formulation containing spores of *Trichoderma asperellum* within 10 and 30 min after inoculation, were free of disease when stored at 28 °C for 7 days.

The major postharvest problems of pineapple are disease and chilling injury (Paull, 1992). The fungus penetrates through wounds, caused by de-crowning, that occur during postharvest handling (Figure 2.7). Likhitekaraj *et al.,* 2007 reported that three species of microorganism were detected on the cutting end of crown e.g. *Penicillium* sp., *Phoma* sp. and *Alternaria* sp. Several methods have been developed for avoiding postharvest diseases and microbial attacks of pineapple, including fumigants (methyl

bromide), chemical disinfection such as dipping in triabendazole or benomyl (Kader *et al.*, 2009).

Figure 2.7 Fungus symptoms on the de-crowned pineapple fruit.

2.5 Electrolyzed oxidizing water

Elictrolyzed oxidizing (EO) water was developed in Japan (Izumi, 1999). Its advantages over other cleaning agents include effective disinfection, easy operation, low cost and low environmentally impact (Huang *et al.*, 2007; Abadias *et al.*, 2008). It is produced by the electrolysis of a dilute solution of sodium chloride passing an electric current through an ion exchange membrane which separates the anode from the cathode. By subjecting the electrodes to direct current voltage, negatively charged ions such as hydroxide (OH^-) and chloride (Cl^-) in the salt solution move to the anode to release electrons and become oxygen gas (O_2), chlorine gas (Cl_2), hypochlorous acid (HOCl) and hydrochloric acid (HCl). Positively charged ions such as hydrogen (H^+) and sodium (Na^+) move to the cathode to take up electrons and become hydrogen gas (H_2) and sodium hydroxide (NaOH)(Hsu, 2005). As a result, two types of water possessing different characteristics are generated. An electrolyzed basic solution (pH > 11 and oxidation reduction potential (ORP) < -800 mV) is produced at the cathode side. It has a strong reducing potential and can be used as a cleaning solution. An electrolyzed acid

solution (pH < 2.7, ORP > 1100 mV and presence of hypochlorous acid) is produced at the anode side. This solution has a strong oxidation potential and bactericidal effect that can be used as a disinfectant (Hsu, 2005). The principle of producing electrolyzed water is shown in the figure 2.8 with following:

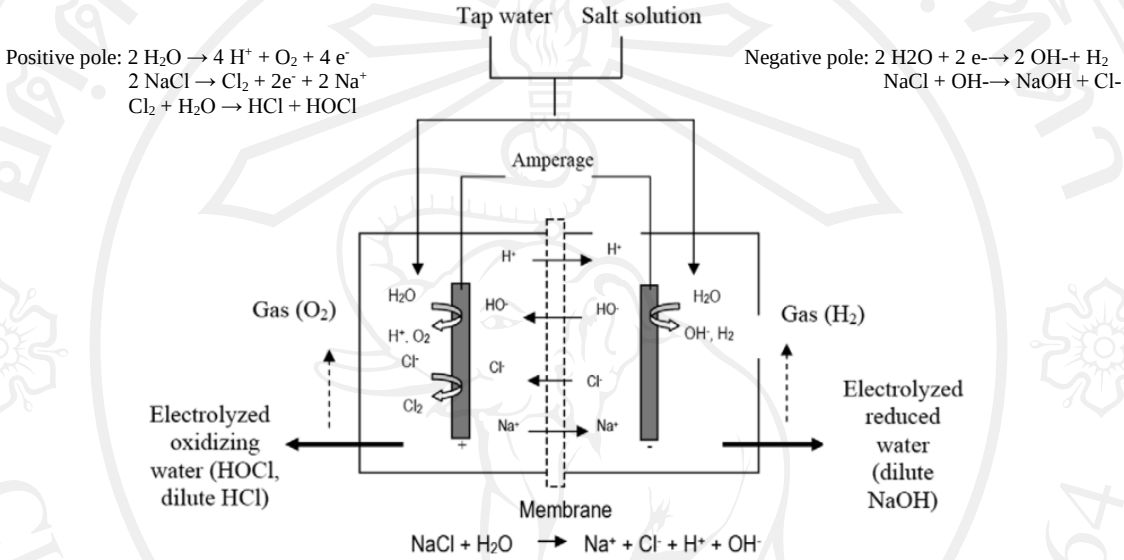

Figure 2.8 Schematics of electrolyzed water generator and produced compounds (Huang *et al.*, 2007)

2.5.1 Advantages of EO water

EO waters are completely non-toxic cleaning and sanitizing solutions that replace all chemical detergents and biocides. The advantages of these solutions are its safety. EO water is a strong acid, but contrary to HCl, it acts different, in that it is not corrosive to skin, mucous membrane, or organic material. When EO water makes contact with organic matter, or is diluted by tap water, it becomes ordinary water again. Thus, EO water has less adverse impact on the environment and users' health. Compared with other conventional disinfecting techniques, EO water reduces cleaning times, is easy to handle, has very few side effects, is relatively cheap and compared to NaClO it has a strong bactericidal effect against pathogens and spoilage microorganism, due to high ORP. Chemicals used for cleaning and disinfecting are expensive. Once the initial

capital investment is made to purchase an EO water generator, the only operating expenses are water, salt and electricity to run the unit (Huang *et al.*, 2007). Disinfection with EO water is more convenient than conventional chlorine disinfection for the following reasons (AL-Haq, 2005):

1) It can be produced on-site.

2) It is produced by simple electrolysis using pure water with no added chemicals except for a dilute salt solution (NaCl or KCl or MgCl).

3) Its use reduces the cost and hazards associated with the handling, transportation, and storage of concentrated chlorine solution.

4) It is more environment-friendly. In some machines, if the pH is set lower than 5.0, chlorine gas is produced in addition to other species such as HOCl and OCl^- ions.

5) To reduce health concerns about the use of chlorinated water, EO water can be modified to reduce the available chlorine concentration while maintaining its effectiveness against microbes.

6) It reverts to normal water after use, without releasing large amounts of harmful gases such as chlorine.

7) After the initial cost of the electrolysis apparatus, operational expenses are minimal. Thus, the use of electronically generated chlorine is quite cost-effective.

8) As a non-thermal method, the use of EO water does not result in changes in ingredients, texture, scent, flavor, etc., which are brought about by heat-treatment.

9) The cytotoxicity of EO water is less than that of a conventional disinfectant.

2.5.2 Disadvantages of EO water

The main disadvantage of EO water is that the solution rapidly loses its antimicrobial activity if EO water is not continuously supplied with H^+, HOCl and Cl_2 by electrolysis (Artés *et al.*, 2009). EO water is a more capable disinfectant than conventional chemical disinfectants. However, chlorine gas emission, metal corrosion, and synthetic wax degradation, due to its strong acidity and free chlorine content, are common risks. During the EO water generation process, chlorine ions are generated, and thus chlorine gas is emitted. For extracting the emitted gas it is necessary to use a standard-type extractor fan (Huang *et al.*, 2007; AL-Haq, 2005).

1) It may rust some metals.

2) Its effectiveness is reduced by the presence of protein because chlorine reacts with protein.

3) Among water-electrolyzing machines, some models, if operated at pH < 5, produce pungent chlorine gas that causes discomfort for the operator.

4) The initial purchase of the equipment may be costly.

5) With time, the bactericidal activity of EO water is reduced due to chlorine loss.

6) EO water contains free chlorine, which can be phytotoxic to plants and damage plant tissue.

2.5.3 Application of EO water for postharvest disease control

Many studies have been conducted in evaluating the bactericidal activity of EO water and the application of EO water at postharvest stage in the agriculture is presented in following;

1) Postharvest sanitation management inactivation pathogens

The use of EO water as disinfectants for food processing equipment has been studied by several researchers. Tomás-Callejas *et al*. (2011) reported that washing fresh-cut baby mizuna leaves with EO water showed an inhibitory effect on natural microflora growth compared to a control. It is may prove to be effective for postharvest sanitation of fruit surfaces prior to packaging and may increase the shelf life of the peaches and grapes fruit in commercial settings(Guentzel *et al.,* 2010). Total aerobic bacteria, mold and yeast populations on slices carrots were reduced after EO water (23 mg/L available chlorine, pH at 5.5) treatment. Treatments reduced the total aerobic bacteria by 2.2 $\log_{10}$ CFU/g and molds and yeasts by >1.9 $\log_{10}$ CFU/g compared with tap water treatment. The use of EO water is suggested as an effective disinfection method for fresh cut carrots with low available chlorine (Koide *et al.*, 2011). Further, it has been reported that the strongest bactericidal efficacy among all the sanitizers (aqueous ozone, 1% citric acid and sodium hypochlorite solution) on oyster mushroom, the inactivation of natural microflora (total aerobic bacteria counts, yeast and molds) by reductions of 1.35, 1.08 and 1.90-2.16 log CFU/g after 3 min treatment at room temperature (23 ± 2 °C), respectively (Ding *et al.*, 2011).

2) Postharvest application to control fungal decay

Although postharvest losses cause by fungus may be reduced with chemical disinfection but those treatments have toxicological risks and may cause environmental pollution. Increasing concern about pesticides in the environment, potential worker safety issues, and fungicide resistance indicate the need for alternative disease control measures (LaMondia and Douglas, 1979). EO water is a potential alternative to fungicide in the control of postharvest diseases. It has wide fungicidal activity, which may facilitate its

use as a contact fungicide on aerial plant surfaces and for general sanitation in greenhouses (Al-Haq *et al.,* 2005). The inactivated mechanism of EO water on fungi might be because of °OH exists in EO water can damage the normal morphological structure of conidia. The normal function of conidium cell wall and membrane has also been damaged closely relation with °OH. These damages lead to the leakage of K^+ and Mg^{2+} ions. Finally bring to the conidia lose their normal function Xiong *et al*. (2010).

Some studies have reported that EO water could be used as an alternative to conventional fungicides for controlling plant fungal disease. According to Yamaki (1998) who used EO water for controlling powdery mildew in cucumber and found that it apparently reduced powdery mildew for about two weeks from 18 days after planting. He also found that fungal decay was delayed for about two days in peaches treated with EO water, while control peaches started to decay the day after harvest. He reported that disease incidence was 70% in the control and 20% among EO water -treated peaches.

Buck *et al*. (2002) reported that electrolyzed water used to treat 22 fungal species, significantly reduced growth of the thin-walled species (e.g., *Botrytis cinerea* and *Monilinia sp.*) within 30 seconds. Additionally, it significantly reduced growth of the thicker-walled, pigmented fungi (*Curvularia* sp. and *Helminthosporium* sp.) within 2 minutes or less. Moreover, Whangchai *et al*. (2009) found that EO water treatment of tangerine cv. Sai Nam Pung, at a free chlorine concentration of 215 ppm for 120 and 240 seconds, completely inhibited the growth and development of *Penicillium digitatum*. Moreover, washing orange with EO water with continuous ozone exposure for 2 h/day significantly controlled *P. digitatum* disease during storage (Whangchai *et al.,* 2010). Postharvest dip and daily spray via integration of near-neutral EO water with misting operations of commercial retail settings may reduce fungal growth of *B. cinerea* and *M. fructicola* on surfaces and increase the shelf life of peach and grape has also been demonstrated (Guentzel *et al.,* 2010).

3) Postharvest application to control bacterial decay

In general, bacteria grow in a pH range of 4-9. Aerobic bacteria grow mostly at a ORP range + 200 mV to 800 mV, while anaerobic bacteria grow well at -700 to + 200 mV. A higher ORP of EO water inactivates bacteria because this potential causes modification of metabolic fluxes and ATP production, probably due to the change in the electron flow in cells. Low pH may sensitize the outer membrane of bacterial cells to the entry of HOCl. The HOCl is the most active of the chlorine compounds, and appears to kill the microbial cell by inhibiting glucose oxidation. It oxidizes meanly sulfhydryl groups of certain enzymes which are important in carbohydrate metabolism of bacteria (Huang *et al.*, 2007).

Many research reported that EO water exhibited strong antibacterial activity against a number of food-borne pathogens in fruit and vegetable. Kim *et al.,* (2000) reported EO water containing 10 mg/l of residual chlorine was very effective in reducing the populations of *E. coli* O157: H7, *L. monocytogenes* and *B. cereus* vegetative cells to undetectable levels after treated for 60 second. Abadías *et al*. (2008) also demonstrated that the bactericidal activity of diluted NEW (50 mg/l free chlorine, pH 8.6) against *E. coli* O157.H7, *Salmonella*, *Listeria innocua* and *Erwinia carotovora* on fresh-cut lettuce was similar to that with NaClO (120 mg l^{-1}). Tomato fruit peels after treated with EO water resulted in a decline of bacteria such as *Escherichia coli* 0157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, without any effect on the environment (Deza *et al*., 2003). Hung *et al*. (2010) reported that EO water treatment of strawberries and broccoli significantly reduced the *E. coli* 0157:H7 counts Paola *et al*. (2005) found that washing lettuce with EO water for 5 minutes significantly inhibited the growth of *L. monocytogenes*. Guentzel *et al*. (2008) also reported that EO water could be reduced bacterial populations on spinach and lettuce after used as a microbial decontamination agent and as a rinse treatment. The effect of EO water in reducing the growth of pathogenic microorganisms and spoilage organism on fresh fruits and vegetables has been investigated. It significantly reduces populations of *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Listeria monocytogenes* from the surfaces of lettuce and

spinach, with increasing time of exposure from 15 s to 5 min at 22 ± 2 ºC (Park *et al.*, 2008).

Recent studies by Issa-Zacharia *et al*. (2010) have been demonstrated that EO water treatment significantly reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by ≥ 2.5 log CFU/g relative to un-treated samples and the population of *E.coli* and *Salmonella* spp. were also significantly reduced by ≥ 2.7 log CFU/g and ≥ 2.9 log CFU/g, respectively from each of tested samples of Chinese celery, lettuce and daikon sprouts following a EO water treatment. It is provided effective inactivation of *E. coli, L. innocua* and *S. choleraesuis* on apple slices. Comparing the electrolyzed water effect to that of chlorination, it seems that the amount of free chlorine used by the fresh-cut industry for disinfection of fresh-cut produce could be reduced while achieving the same or better disinfection results (Graca *et al.*, 2011).

2.6 Ultrasonic wave

US is a form of energy generated by sound waves of high frequencies that the human cannot be detected by ear, i.e. above 16 kHz (Jayasooriya *et al.*, 2004). In recent years, the food industry has discovered that ultrasonic has a variety of applications in the processing (Piyasena *et al.*, 2003). US technology has a bactericidal effect, caused by the occurrence of the cavitation phenomenon, which consists of the formation, growth, and collapse of air bubbles. These bubbles generate localized mechanical and chemical energies that are capable of inactivating microorganisms (Adekunte *et al.*, 2010; Gogate & Kabadi, 2009; Piyasena *et al*., 2003; Valero *et al.*, 2007). The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals (Butz and Tauscher, 2002). During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala *et al.*, 1995). These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, causing the bubble to expand. A point is reached where

the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble; therefore, rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching up to 5500 °C and 50,000 kPa. The pressure changes resulting from these implosions are the main bactericidal effect in ultrasound. The hot zones can kill some bacteria, but they are much localized and do not affect a large enough area (Piyasena *et al*., 2003). The ultrasonic cavitation cycle are summarize in Figure 2.9. The cavitation threshold of a medium (that is, the minimum oscillation of pressure that is required to produce cavitation) is determined by a number of factors. Among these are dissolved gas, hydrostatic pressure, specific heat of the liquid and the gas in the bubble, and the tensile strength of the liquid. Another extremely important variable is temperature, which is inversely proportional to cavitation threshold. The ultrasonic frequency used must be under 2.5 MHz, as cavitation will not occur above that level (Alliger, 1975).

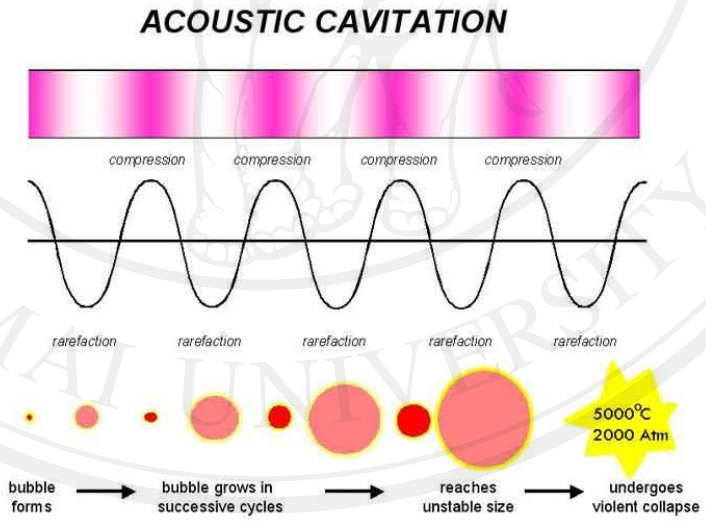

Figure 2.9 Ultrasonic cavitation cycle (http://www.electrowave.org/Cavitation.html)

2.7 Combined treatments

Ultrasound has also been used in combination with organic acid or other sanitizer. Inactivation of *Saccharomyce*s with a combination of heat and ultrasound has been found to be almost independent of pH (Guerrero *et al.*, 2001). Salleh-Mack and Roberts (2007) reported that combination of ultrasound and organic acid significantly reduced *E. coli* in simulated juice. Another continuous system working in combination with steam injection has been shown to afford between 1.5- and 4-fold higher inactivation rates of *E. coli* and *Lactobacillus acidophilus* in several liquid foods such as milk or fruit juices (Zenker *et al.*, 2003). Combined treatment with ultrasound and 2% organic acid for 5 min shown that the maximum reductions of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* were 2.75, 3.18, and 2.87 log CFU/g, respectively (Sagong *et al*., 2011). A similar approach by Scouten and Beuchat (2002) to decontaminate alfalfa seeds inoculated with *Salmonella* or *E. coli* O157 showed that the combined treatments of ultrasound and $Ca(OH)_2$ could be an alternative to chlorine treatments to avoid contamination in the sprouts. A greater reduction in the number of cells on the surface of cherry tomatoes, including *Salmonella* has been achieved by using ultrasound combined with peracetic acid (Brilhante São José *et al.*, 2012). One to 1.5 log reductions of *Salmonella* contaminating poultry surfaces have been achieved by using ultrasonic irradiation alone but up to 4 log reductions were achieved by combining the same irradiation intensities with minimal (0.5 ppm) chlorine concentrations (Lillard, 1993).

Further, salicylic acid (SA) combined with ultrasound treatment was more effective in inhibiting fungal decay during storage than the SA treatment alone. Using ultrasonic waves (40 kHz, 10 min) and salicylic acid (SA) (0.05 mM) on peach fruit resulted in significant control of *Penicillium expansum* which causes blue mold. Salicylic acid combined with ultrasound treatment was more effective in inhibiting fungal decay and increased the activities of defense enzymes such as chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase(Yang *et al.*, 2011). In the same way, Cao *et al*. (2010a) used ultrasound treatment on strawberries at a frequency of 0, 25, 28, 40 or 59 kHz at 20°C for 10 min, and then stored at 5°C for 8

days. The treatment significantly reduced decay incidence and numbers of microorganism without affecting the quality of strawberry. In addition, Cao *et al*. (2010b) found that ultrasonic treatment in strawberry at a power of 250W for 9.8 minutes during storage for 8 days at 5◦C effectively inhibited decay incidence, extended shelf-life and maintained quality of strawberry fruit. Chen and Zhu (2011) also reported that the combination of simultaneous ClO_2 (40 mgl^{-1}) and ultrasonic (100W)treatments for 10 min maintained high storage quality of postharvest plum fruit during storage and prolonged the shelf-life for 25 days.

2.8 Plant defense responses during pathogen attacks

Disease is any physiological abnormality or significant disruption in the “normal” health of a plant. Plants protect themselves from various stresses such as pathogen attacks, wounding, and application of chemicals including phytohormone and heavy metals, air pollutants like ozone, ultraviolet rays, and harsh growing conditions. These protective reactions are known as "defense responses" of higher plants (Figure 2.10), and the proteins actively synthesized in accordance with this reaction are called "defense-related proteins"(Bowles, 1990). In particular, protective plant proteins specifically induced in pathological or related situations have been intensively studied from an agricultural perspective and are called "pathogenesis-related proteins" (PR proteins). Enzymes indispensable for biosynthesizing low molecular weight antibiotics (phytoalexin), isoflavone reductase, can also be considered to be defense-related proteins. Plants often wait until pathogens are detected before producing toxic chemicals or defense-related proteins because of the high energy costs and nutrient requirements associated with their production and maintenance (Freeman and Beattie, 2008).

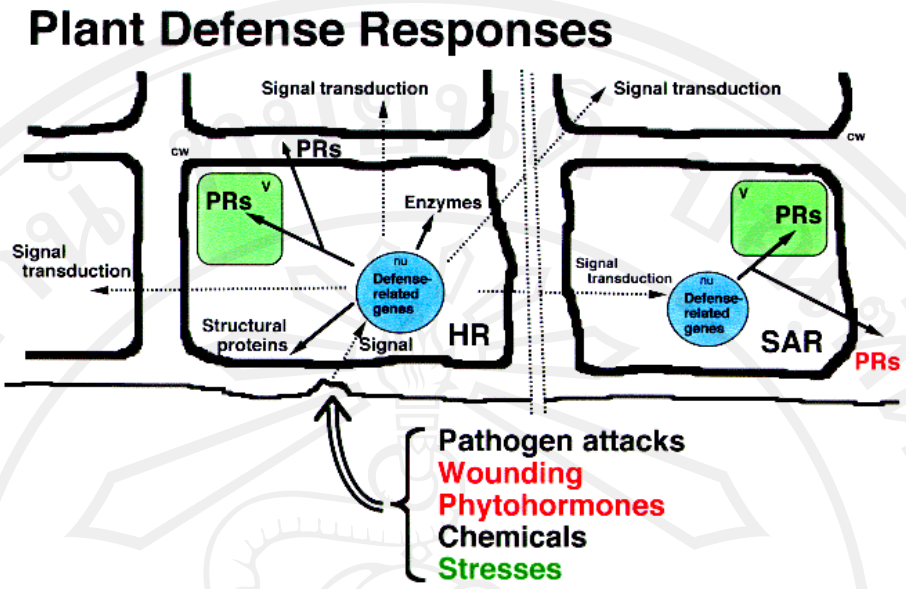

Figure 2.10 Schematic of plant defense response (source:http://dmd.nihs.go.jp/latex/defense-e.html).

2.8.1 Physical defenses

Plant tissues contain pro-formed structural barriers that help limit pathogen attachment, invasion, and infection. The cell wall is a major line of defense against fungal and bacterial pathogens. It provides an excellent structural barrier that also incorporates a wide variety of chemical defenses that can be rapidly activated when the cell detects the presence of potential pathogens. The cell wall of higher plants has been divided traditionally into three structural regions: middle lamella, primary wall, and secondary wall. The middle lamella contains pectin. Pectins form hydrated gels that help "cement" neighboring cells together and regulate the water content of the wall. The primary wall is consists of microfibrils, which are cellulose chains. It is composed of pectin, cellulose, hemicellulose, and proteins. This layer is capable of expansion during growth. The secondary wall is the wall portion added after cell elongation is complete. The cell becomes much less flexible and finally almost inelastic after the deposition of the secondary wall. The most abundant constituent of the secondary wall is cellulose (Figure 2.11) (O'Neill and York, 2003).

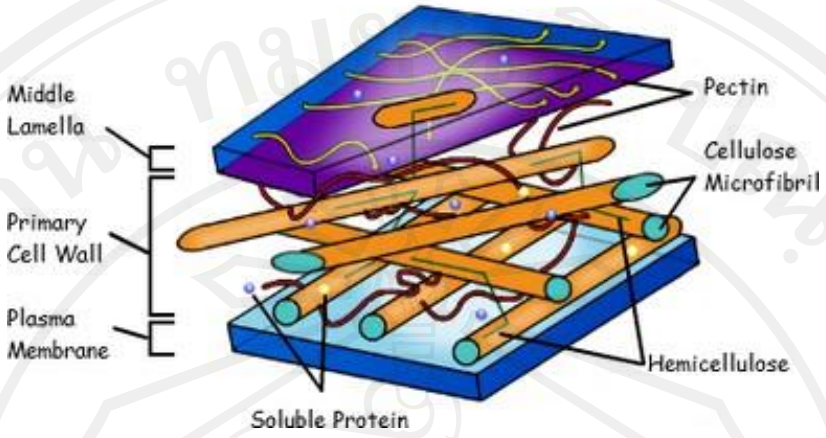

Figure 2.11 Plant cell wall components in the mature cell. (Adapted from McCann and Roberts, 1991).

All plant parts exposed to the atmosphere are coated with layers of lipid material that reduce water loss and help block the entry of pathogenic fungi and bacteria. The principal types of coatings are cutin, suberin, and waxes. Cutin, suberin, and their associated waxes form barriers, between the plant and its environment, that function to keep water in and pathogens out (Jeffree 1996). The cuticle and suberized tissues are both important in excluding fungi and bacteria, although they do not appear to be as important in pathogen resistance as some other defenses, such as phytoalexin production and the hypersensitive response. Many fungi penetrate the plant surface directly by mechanical means. Others produce cutinase, an enzyme that hydrolyzes cutin and thus facilitates entry into the plant (Lincoln and Eduardo, 2010).

2.8.2 Chemical Defenses

Plant chemicals can be divided into two major categories: primary metabolites and secondary metabolites. Primary metabolites are substances produced by all plant cells that are directly involved in growth, development, or reproduction. Examples include sugars, proteins, amino acids, and nucleic acids. Secondary metabolites are not directly involved in growth or reproduction but they are often involved with plant defense. These compounds usually belong to one of three large chemical classes: terpenoids, phenolics, and alkaloids. Phenolic compounds are an important group of secondary

metabolites, which are synthesized by plants as a result of plant adaptation to biotic and abiotic stress conditions (infection, wounding, water stress, cold stress, high visible light). Protective phenylpropanoid metabolism in plants has been well documented (Lincoln and Eduardo, 2010).

2.8.2.1 Plant defense related proteins

Pathogenesis-related (PR) proteins are often induced in response to infection. The synthesis and accumulation of these PR-proteins have long been thought to play an important role in the plant defense response (Ying-Zhang *et al.,* 2003). Van Loon and Van Kammen (1970) showed that a set of proteins is induced in tobacco plants after tobacco mosaic virus infection. PR proteins were shown to be induced not only by pathogens but also by wounding, fungal cell wall elicitors, ethylene, UV light, heavy metals, etc. Well-characterized are glucan and chitin fragments derived from fungal cell walls, fungus-secreted glycoproteins, peptides, and proteins of elicitin family (Münch-Garthoff *et al.,* 1997; Honée *et al.,* 1998). Chitinase (EC 3.2.1.14) and β-1,3-glucanase (EC 3.2.1.39), have been suggested as the key enzymes in catalyse the hydrolysis of chitin and β-1,3-glucan, the main components of fungal cell wall (Figure 2.12). Chitinase play a direct role in plant defense by attacking chitin, a β-1,4-linked polymer of N-acetyl-D glucosamine, a major component of fungal cell walls. While β-1,3-glucanase catalyze hydrolytic cleavage of the 1,3-β-D-glucosidic linkages in β-1,3-glucans. Thus affecting the spread of the invasion and playing an important role in plant defense mechanisms (Broglie *et al.,* 1991).

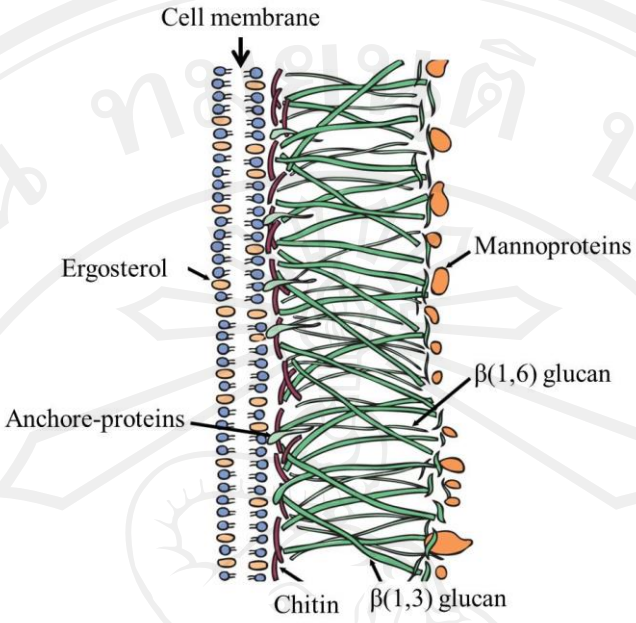

Figure 2.12 Schematic structure of the cell membrane and cell wall fungi (Source: http://elte.prompt.hu/sites/default/files/tananyagok/plants_fungi/index.html.)

2.8.2.2 Plant defense-related enzyme

Plant enzymes are involved in defense reactions against plant pathogens. These include oxidative enzymes such as peroxidase (POD) and polyphenol oxidase (PPO), which catalyse the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko *et al.,* 1993). Other enzymes such as phenylalanine ammonia-lyase (PAL) are involved in phytoalexin or phenolic compound biosynthesis (Bashan *et al.,* 1985; Beaudoin-Eagan and Thorpe, 1985). PAL features in the phenylpropanoid metabolic pathway (Figure 2.13), which is responsible for lignin synthesis (Galis *et al.,* 2006; Ferrareze *et al.,* 2013).

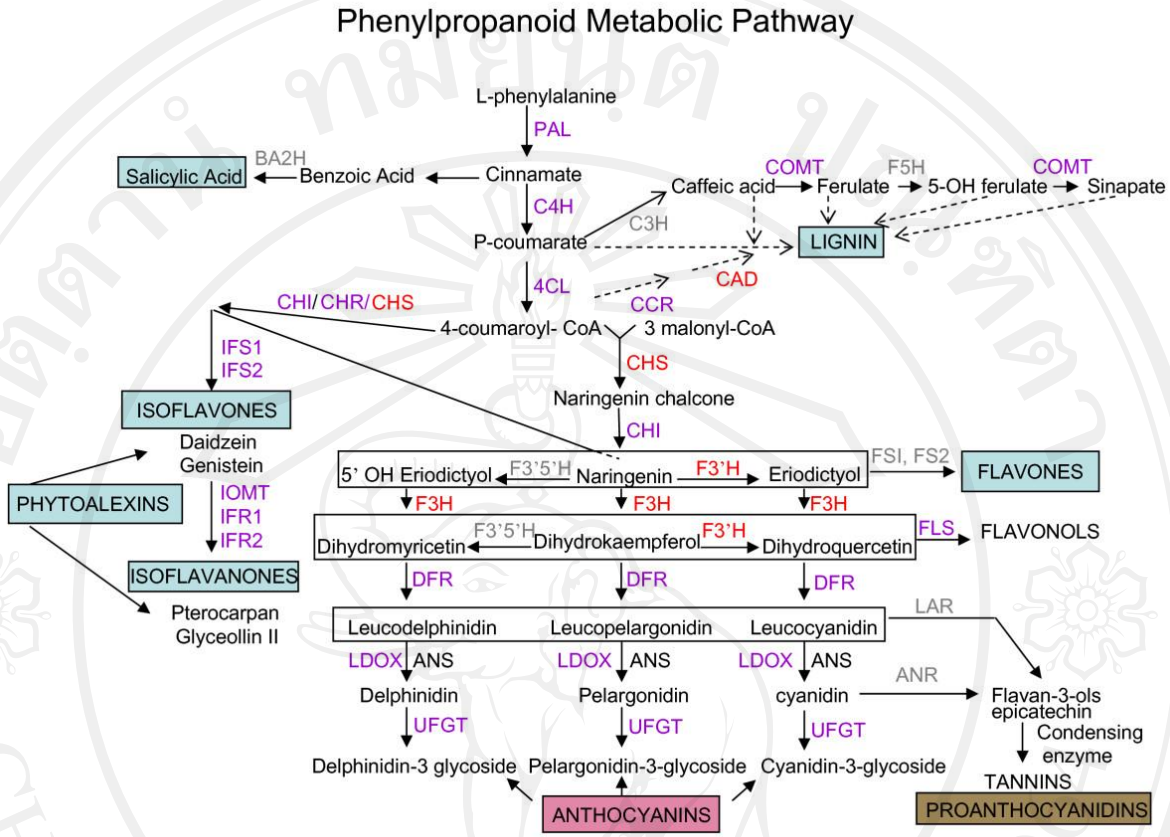

Figure 2.13 Phenylpropanoid metabolic pathway (Zabala *et al*. ,2006).

POD is the enzyme that involve in cross-linking of cell wall components, wound healing and the generation of antifungal metabolites. They are also believed to be involved in removal of H_2O_2, oxidation of toxic reductants as well as defense against pathogen or insect attack (Kozlowska *et al.*, 2001; Cosio and Dunand, 2009). Increased levels of POD activity have been observed in conjunction with both local and systemic disease resistance (Karban *et al.*, 1989; Kombrink and Somssich, 1995). The diverse functions and roles of peroxidases are summarize in Figure 2.14.

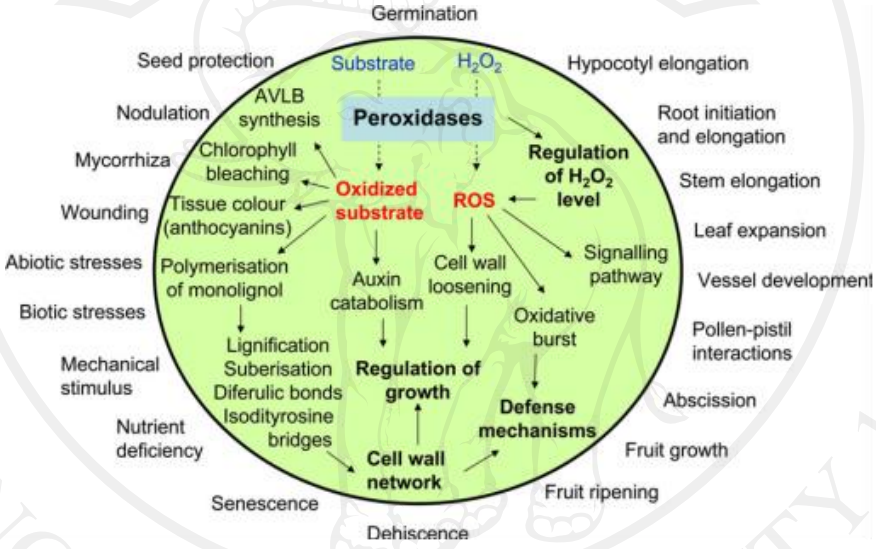

Figure 2.14 The diverse functions and roles of peroxidases (Cosio and Dunand, 2009)

PPO catalyzes the oxygen dependent oxidation of phenolics to quinines (Li and Steffens, 2002). The extent of the damage caused by reactive oxygen species depends to a large extent on the level of coordination among the various scavenging enzymes (Hao *et al.*, 2012). Tobacco anthracnose, the causative agent of which is the hemibiotrophic fungus *Colletotrichum nicotianae* (Lucas, 1965), is a highly destructive pathogen of tobacco seedlings. *C. nicotianae* is classified as a hemibiotroph because it initially establishes a biotrophic interaction with its host before eventually switching to its destructive necrotrophic lifestyle (Shen *et al.*, 2001). Raj *et al*. (2006) found the involvement of PPO in imparting resistance of pearl millet (Penisetum glaucum) to downy mildew. They pointed out that resistant genotypes had elevated levels of PPO, whose formation was rapidly induced following infection, while susceptible cultivars failed to accumulate PPO even after a considerable time. Karthikeyan *et al*. (2006) suggested that the increased level of expression of various isoforms of PPO were implicated in induced defense responses against Ganoderma in coconut. An attempt to present schematically some of the events leading to PPO activity by Mayer (2006) is shown in Figure 2.15.

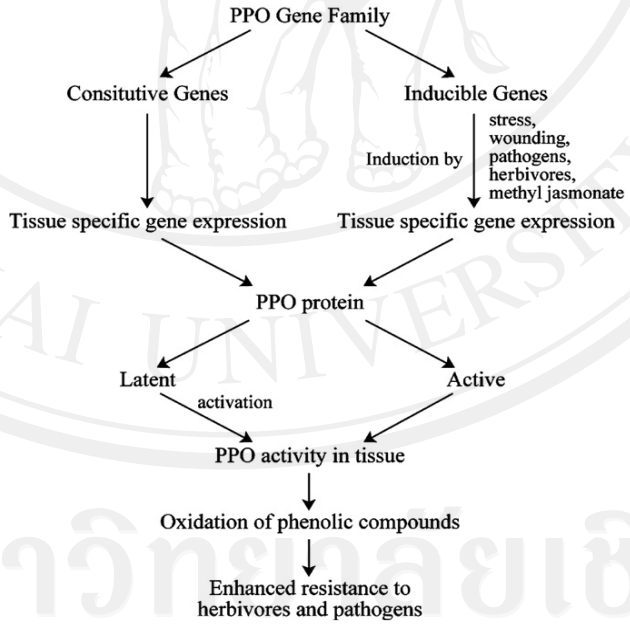

Figure 2.15 Schematically some of the events leading to PPO activity, related to defense mechanism in plant (Mayer, 2006)